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RESEARCH ARTICLE

SOLUBILIZATION OF PHOSPHATE IN LIQUID MEDIUM AND LETTUCE DEVELOPMENT

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ABSTRACT

The objective of this study was to evaluate the efficiency of fungus Aspergillus niger M22 in phosphate solubilization in liquid medium and lettuce development. Both Araxá phosphate and rock residue were added at the dose of 3 g to 100 mL of culture medium yeast extract glucose. The phosphate treatments were inoculated with 1 mL of fungal culture containing 108 UFC mL-1 incubated at 28° C under 190-rpm shaking, for eight days. Both pH and P content were evaluated at the end of the incubation period. To evaluate the potential of M22 in solubilizing nutrients for lettuce plants, combinations of pellets of Aspergillus nigers isolate, of Araxá apatite rock and fertilizer were used as treatments. Isolate M22 presented a higher amount of P soluble in Araxá phosphate than in phosphate rock residue. M22 increased about four times the amount of P soluble in Araxá phosphate medium in relation to the control and about three times in the medium with phosphate rock residue. Isolate M22 demonstrated capacity of solubilizing phosphates. Araxá apatite rock possesses potential to be utilized as a source of phosphorus and potassium.

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INTRODUCTION

Brazil is a great grain producer with some four million of annual tons of consumption of phosphate fertilizers and 50% are imported. Essential to plants, phosphorus is regarded as a nutrient limiting to plant production with limited availability in tropical soils. According to Malavolta (2006), this element is undoubtedly the one which limits the most the plant production in Brazil and, the rise of its availability in order to overcome the barrier imposed by the "hunger of the soil" for this nutrient, is one of the great challenges in the soil fertility management.

Thus, high doses of phosphate fertilizers are necessary for the crops to obtain high yields. It must be taken into account, therefore, that fertilization is one of the most compelling production costs, reducing the profit margin received by farmers. Many microorganisms solubilize different forms of inorganic phosphates.

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The highest solubilization of inorganic phosphate occurs in plants' rhizosphere due to the acidification owing to the release of protons by the plant and of organic acids by microorganisms, these ones being named inorganic phosphate-solubilizing microorganisms (MSFI) (Rodríguez and Fraga, 1999; Tironi *et al.*, 2009). The organisms' solubilizing activity has arisen the interest of both researchers and farmers for the development and application of microbial inoculants (Dalcin, 2008). The inoculation of microorganisms or the management of their populations have been suggested as a way to replace or reduce the use of soluble phosphate fertilizers through a better use of natural phosphates or the phosphates added to soil and formed by the application of soluble fertilizers. According to Bottomley (2005), soil microorganisms influence directly the fertility and plant yield by means of nutrient cycling, suppression of plant pathogens, plant hormone production and in addition, the capacity of metabolizing agrotoxics. Also, Das *et al.* (2003) and Tótola and Chaer (2002) stressed that the activities of microorganisms are involved in the biogeochemical cycle, since they influence directly nutrient availability and elucidate better the changes of the functioning of the ecosystem soil (Reis *et al.*, 2008). Better understanding of the capacity and efficiency of microorganisms to solubilize different phosphates may lead to the selection of isolates of high potential of use for inoculation in plants (Souchie *et al.*, 2005). The objective of this study was evaluating the efficiency of fungal isolate *Aspergillus niger* M22 as to its capacity of solubilizing phosphates in liquid medium as to the availability of P and K in lettuce development.

MATERIALS AND METHODS

Evaluation of the solubilization capacity by strain M22 of Aspergillus *niger* was performed in EPAMIG Microbiology Laboratory in the Federal University of Lavras (Universidade Federal de Lavras) -MG, Brazil. The utilized fungus was *Aspergillus niger* M22, isolated from coffee collected on the "Ponte do Funil" farm in the town of Perdões - MG, selected for presenting a high capacity of solubilizing phosphates. The fungus is stored in the collection of EPAMIG Microbiology Laboratory. Araxá phosphate rocks (14 g kg^{-1} of P) and phosphate rock residue (9.15% of P) were used in phosphate solubilization. Araxá phosphate (14 g kg-1 of P) and rock residue (P 9.15%) were added at dose of 3g directly to 100 mL of liquid GL culture medium (glucose, yeast extract). In each balloon (250 mL) with 100 ml of culture medium with the treatments of phosphate, 1 mL of culture of isolate M 22 containing 10^8 CFU mL-1, incubated at 28 ° C, under shaking of 190 rpm for eight days was inoculated. The two sources of phosphorus (Araxá phosphate and rock residue) were tested with the culture medium utilized for growth of isolate M 22 with isolate M 22 in addition to two control treatments (culture medium and culture medium + M22), amounting six treatments in three replicates. The efficiency of solubilization was determined pH and content of soluble P at the end of the incubation period was evaluated. The data were submitted the analysis of variance and the means were compared by the Tukey test at 5% of probability by utilizing the SISVAR program. To evaluate the potential of microorganism M22 to solubilize nutrients for lettuce plants, an experiment was conducted in greenhouse by utilizing combinations of pellets of the isolate of *Aspergillus niger*, of Araxá apatite rock and fertilizer. In the growing of the lettuce seedlings variety crisphead, a three-liter pot with organic substrate was used. The fertilization of the substrate of the lettuce plants was with 10g of NPK 4 14 8 and 10g of Araxá apatite rock powder with the combinations of 5, 10 and 15g of pellet of M22. The control was the use of the pot only with the organic substrate. Sixty days after germination of the lettuce (lettuce seedlings of the variety crisphead), the shoot of the plants was collected and dried at 75 ºC for evaluation of the weight of the dry matter and determination of the content of phosphorus and potassium. The determination of phosphorus and potassium was done in the atomic absorption apparatus (Atomic absorption spectrophotometer model GBC 932 AA).

RESULTS AND DISCUSSION

In liquid medium, the isolate of *Aspergillus niger* presented increased amount of soluble P in Araxá phosphate than with the phosphate rock residue. Isolate M22 reduced the pH of the liquid medium both with Araxá phosphate and rock residue in relation to the control without inoculation (Figure 1). In general, the medium with the rock residue was less acidified than that with Araxá apatite, though that poorer acidification had occurred in the control treatment e of the phosphate source.

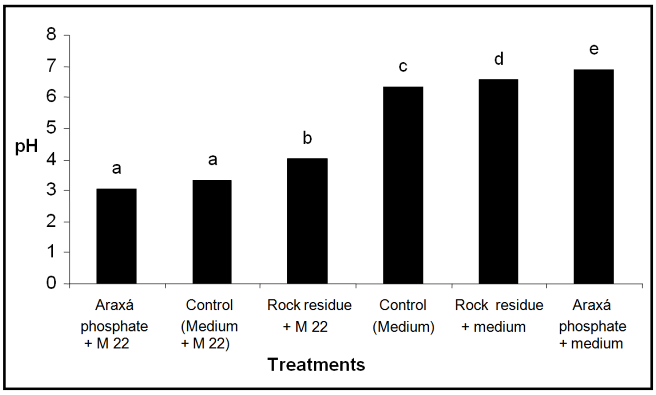

Figure 1. Effect of isolate M 22 on the medium pH

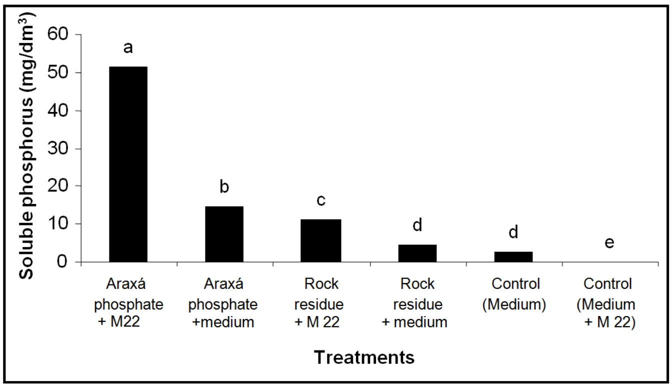

Figure 2. Effect of isolate M 22 on phosphorus solubilization

The study by Chuang *et al.* (2007) pointed out the significant and positive correlation between phosphate and acidity. For solubilization to exist there is need of the production of acids. That same mechanism was reported in the literature of Vassilev *et al.* (2006), citric acid being regarded as a strong solubilizing agent. The phosphorus solubilization resuted from decreased pH in the two sorts of rocks studied. According to Barroso and Nahas (2005) by increasing titrable acidity occurs decrease in the values of final pH and an increase of titrable acidity or a decrease in the values of final pH corresponds to an increase of the content of soluble phosphate. In the work by Barroso *et al.*, (2008) the efficiency of the solubilization of FePO4 by *Aspergillus niger* in culture medium with different sources of carbon and nitrogen, had the production of acids as the main mechanism of solublization of FePO4 on the basis of the, com correlation between phosphate production and acidity. Concerning the solubilization of phosphate, it was found that Araxá phosphate in the presence of isolate M 22 stood out from the other treatments (Figure 2). The strain of M22, on the average, increased about four times the amount of soluble P in the medium with Araxá phosphate in relation to the control and about three times that of soluble P in the medium with phosphate rock residue. According to Souchie *et al.* (2005), the fungal isolates promoted the double of the P solubilization in the medium with Araxá apatite in relation to bacteria and, on average, more than seven times in the medium with AlPO4, which evidences increased production of acidic metabolites by those microorganisms. This work shows the efficiency of Araxá apatite rock in making phosphate available. Filho *et al.* (2002) evaluated the solubilization of natural phosphates by microorganisms and the contents of P present in the GSE medium after incubation were higher than in Anitápolis phosphate, followed by Araxá phosphate and last, that of Catalão. The differences were related with the composition of the phosphate with the content of CO_2 and, or with the presence of inorganic nutrients which accompany the rock. In the control treatment with the culture medium, a small amount of soluble phosphorus was found, by comparing the control treatment with the medium and inoculum, this had no soluble phosphorus, possibly the utilized Gl medium which has yeast extract had a minimum amount of phosphorus which was utilized by M22 for that reason, the control of medium with the fungus was null. According to Santucci *et al.* (2003) yeast extract contains all the autolyzed soluble material (self-digestion of cells), including proteins, peptides, free aminoacids, nucleotides, vitamins, oligosaccharides and minerals, including macroelements (Ca, P, Mg, K, Na, Al and Fe) and microelements (Mn, Cu, B, Zn, Mo, Cd, Cr, Ni, Pb, Si and Se); carbohydrates between 25-35%. Taking into consideration the general means of dry weight was the rock with the pellet which provided the greatest development of the plants, standing for an increase of 210 % in the dry matter weight in relation to the control (Table 1).

Table 1. Dry matter (g), Phosphorus (mg/kg) and Potassium (mg/kg) in the shoot of lettuce plants (A.) grown in organic substrate inoculated with pellet (P.) of M22, Araxá apatite rock and fertilizer

Treatments with lettuce (L.)	Dry weight	% P	% K
L. Control (nothing)	0,83 i	0,42 j	2,41 m
L. Rock	3,67 h	0,47 i	2,83 i
L. Fertilizer	4,91 g	0,52 h	3,11 g
L. Pellet 5g	5,02 g	0,61 g	2,72 l
L. Pellet 10g	7,84 f	0,65 h	2,79 j
L. Pellet 15g	8,36 d	0,70 d	2,86 h
L. Fertilizer P. 5g	6,59 e	0,64 f	3,22 f
L. Fertilizer P. 10g	8,54 d	0,67 g	3,30 e
L. Fertilizer P. 15g	9,24 c	0,73 c	3,40 d
L. Rock P. 5g	9,52 c	0,75 c	3,95 c
L. Rock P. 10g	20,13 b	0,76 b	4,03 b
L. Rock P. 15g	22,79 a	0,80 a	4,08 a

Means followed by the same small letter in the column do not differ from each other by Tukey's test at 5% of probability.

The treatment with addition of rock together with 15g of pellet provided the greatest weight of lettuce plants, differing statistically from the other treatments. Use of the rock with 5g of pellet did not differ from the treatment with fertilizer and 15g de pellet pointing out the efficiency of the pellet in phosphorus solubilization of Araxá apatite rock. The application of the pellet at the dosages of 5, 10 and 15g resulted into increased dry matter of the lettuce plants when compared with the application of the fertilizer or the rock singly. As organic substrate was utilized in the development of lettuce, the isolated pellet may have solubilized and made the nutrients existing in the substrate available. Works report the efficiency of *Aspergillus niger* in the solubilization of soil phosphate, increasing the availability of this nutrient essential to the growth of plants, resulting into increased dry weight. In study of selection of microorganisms promoting nutrient bioavailability, Dalcin (2008) found that biotite was the rock which provided greater growth of the plants, standing for in increase of 207 % in the dry matter weight in relation to the control. In the work by Lana *et al.* (2004) with lettuce production concerning the use of different sources of phosphorus reported that in the absence of phosphorus, it had significant reduction in the shoot fresh matter and elevated reduction of the phosphorus contents accumulated in the leaves, standing out the elevated phosphorus requirement of the lettuce. Alves and Filho (2009) using different phosphate-solubilizing isolates in the lettuce seedling production had a decrease in the shoot dry matter production, reaching in some cases to a reduction over 50% of the plants' dry matter relative to the inoculum less control. The choice of the isolate in some cases can affect the dry matter production, for that reason, one must test a greater variety of microorganisms and isolates. As regards the percentage of phosphorus, the results were similar to the ones found for the plants' dry weight. The greatest percentage of phosphorus was found in the treatment with rock and 15g of pellet. There was n increase of 47.5% of phosphorus as compared with the control. The treatment with rock and 5g of pellet did not differ from the treatment of fertilizer and 15g of pellet. Phosphorus content in the treatment with the addition of fertilizer or rock singly was statistically lower when compared with the treatment with the presence of the pellet of *Aspergillus niger*. As well as in the plants' dry weight and percentage of phosphorus, the treatment: rock and 15g of pellet showed the highest percentage of potassium, differing from the other treatments. The mixture of 10g and 5g of pellet with rock as well as in the found in the other evaluated parameters was most effective in making K available in relation to the mixture of fertilizer with the three dosages of the pellet of tested *Aspergillus niger*. The control presented percentage of potassium 59,68 lower than the best treatment (rock and 15g of pellet).

Conclusion

Isolate M22 demonstrated the phosphate-solubilizing capacity. Mechanism and plants and microorganisms can be low cost and adequate technologies to stimulate solubilization and increase the agronomic efficiency of rock phosphate.

Araxá apatite rock possesses potential to be utilized as a source of phosphorus and potassium.

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